conditions of opportunity for copulation, or in females caged with males with sexual intercourse established but pregnancy prevented through partial hysterectomy, or in normal females coexisting with males but with coition prevented by a vaginal "chastity" clip.

3. The resistance to such medication was markedly raised in females which were or recently had been pregnant, but was not brought up to that of the males.

4. Young virgin females developed tolerance to gradually increasing doses of Sodium Pentobarbital about ten times more often than did older virgins. Six young rats recovered permanently from medication every 90 minutes for three days and nights.

5. Small doses of Nostal administered at three- to four-day intervals for several weeks completely prevented delayed deaths from 50 mg./Kg. of Nostal, which killed 83 per cent of the controls.

6. Similar preliminary treatment with Sodium Pentobarbital raised the average resistance of virgin female rats only moderately.

7. Varying conditions of sex life did not alter the average percentage weights of the thyroid, adrenal or pituitary glands. The higher proportion of the latter two glands in the females was greater than usually has been reported for the albino rat.

We are indebted to Miss Lucille M. Mills and to Mr. Donald R. Mathieson for aid in this work.

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A Phytochemical and Histological Study of *Purshia tridentata* (Pursh) D.C.*

By Charles V. Netz, Charles H. Rogers and Glenn L. Jenkins

Purshia tridentata (Pursh) D.C., family Rosaceæ, is a diffusely branched shrub grow-

* Presented to the Scientific Section, A. PH. A., Richmond meeting, 1940.

Abstract of thesis presented to the Graduate Faculty of the University of Minnesota by Charles V. Netz in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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This is a report in a coöperative research project connected with the Indian Medicinal Plant study of the Bureau of Plant Industry, United States Department of Agriculture.

ing to a height of 3–5 feet and bearing clusters of cuneate, 3-lobed, small leaves. The small yellow flowers are not prominent. The fruit is a soft, hairy achene. Synonyms are Antelope Brush and Bitter Brush. The plant grows from the western slope of the Rocky Mountains to the mountains of California and northward.

The plant material (serial number H 496) was collected in the summer of 1938 in Washoe County, Nevada, by James R. Henrichs and was identified by Rogers McVaugh of the Division of Plant Exploration and Introduction, Bureau of Plant Industry of the United States Department of Agriculture, Washington, D. C. It consisted of the much-broken, air-dried, over-ground portion containing very few fruits and flowers.

Decoctions, infusions and poultices of the over-ground portions of this plant have been used for years by the Indians of that region for the treatment of pathological conditions and infections ranging from sore eyes and internal hemorrhage to measles, smallpox and gonorrhea. This investigation was intended to isolate and identify any constituents responsible for the purported curative action obtained by the Indians.

Trimble (1) reported a preliminary investigation of the fruit in which he found a "bitter substance," oily constituents, resins, tannins, mucilages, pectin and phosphates of calcium and magnesium.

EXPERIMENTAL

HISTOLOGICAL

The inner morphology of the stem and leaf of *Purshia tridentata* D.C. was studied by means of transverse and radial- and tangential-longitudinal sections supplemented by a microscopic examination of the elements in the powdered material.

The stem revealed a typical dicotyledonous structure with pith parenchyma filled with tannin, xylem, cambium, phloem and bark, the last containing from one to four layers of cork cells and numerous groups of stone cells. The vessels were arranged diffusely and were characterized by oblique and bordered pits and simple end-wall perforations. Spiral trachea and calcium oxalate rosettes were also present.

The leaf showed epidermis, palisade cells, collenchyma, spiral trachea and calcium oxalate rosettes. The adaxial surface was glabrous whereas the abaxial surface bore two types of uni-cellular,

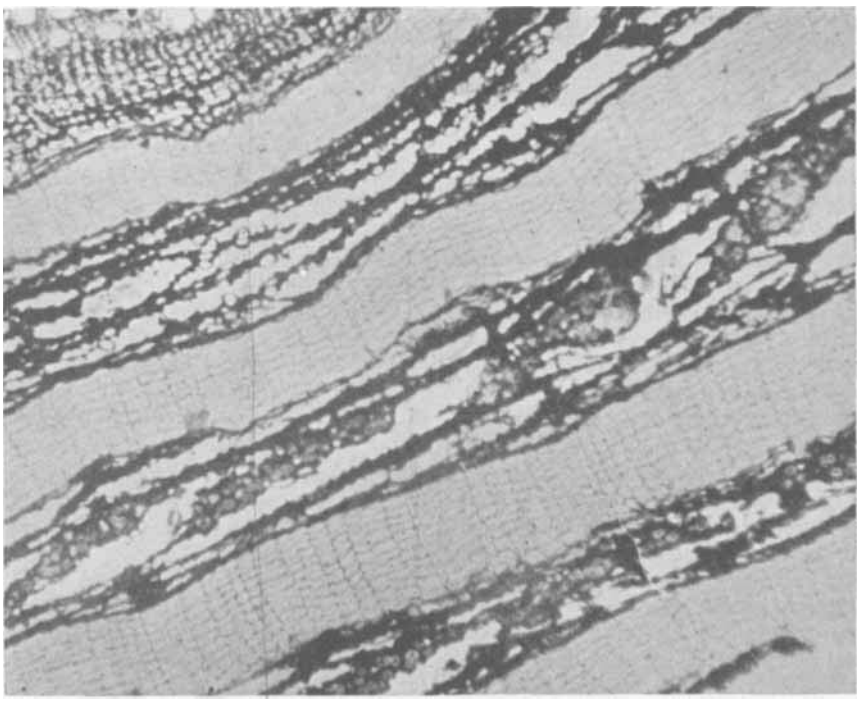

Fig. 1.—Transverse Section of Bark × 100. Stained with Safranin and Haidenhain's Iron-Alum Hæmatoxylin.

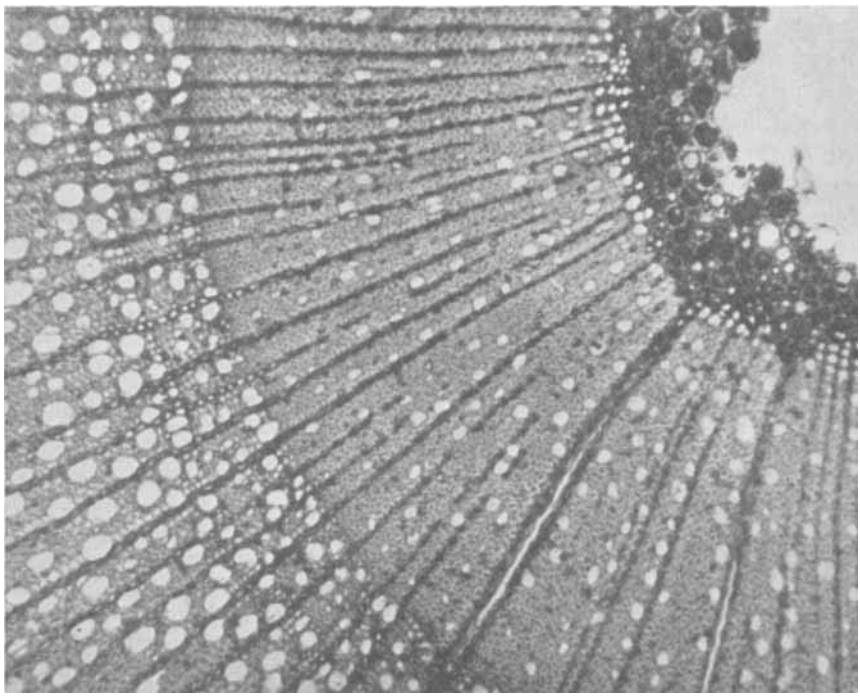

Fig. 2.*—Transverse Section of Wood × 100. Stained with Safranin and Haidenhain's Iron-Alum Hæmatoxylin.

non-glandular trichomes, one short and pointed, the other long and irregularly curled.

PHYTOCHEMICAL

The plant material was reduced to a No. 20 powder and the following constants of the well-mixed powder were determined by U. S. P. methods: total ash 3.12%, acid-insoluble ash 0.39% and moisture 6.91%.

Preliminary Examination.—An infusion of the powdered plant material made with hot water was acid to litmus paper, gave tests for tannins with ferric chloride T.S., and gelatin-salt T.S. and produced a foam indicative of the presence of saponins but gave no test for starch.

Examination of portions of the powder under a microscope showed a few starch grains.

Steam distillation did not yield an appreciable quantity of volatile constituents. Dilution with alcohol of portions of the filtered decoction remaining from the steam distillation produced a stringy, mucilaginous precipitate indicative of mucilage or pectin (2). The filtrate from this precipitate, after being dealcoholized, gave tests (3) which indicated that the tannin present in the plant material was a catechol or catechol-phloroglucinol tannin (Stenhouse-Proctor classification) (4).

Tannin.—A quantitative aqueous extract of the plant material was freed of constituents insoluble in 70% alcohol, and the tannin content was determined by the hide powder method of the A.O.A.C. (5). The tannin content was 6.55%.

Alkaloids, Glycosides and Sucrose.—Extraction of a portion of the powdered plant material with Prollius' fluid and ammonia water and extraction of additional portions with weak hydrochloric acid at 80° and 100° C., respectively, and Prollius' fluid failed to yield alkaloids.

The method of Stas-Otto (6) applied to a portion of the powdered plant material indicated that no glycosides or alkaloids were present.

Tests for cyanogenetic glycosides by methods suggested by Rosenthaler (7), using sodium picrate paper, gave negative results.

The Bourquelot method (8) using yeast and emulsin showed the absence of sucrose but gave indications of the presence of a glycoside.

Protein.—The plant material contained 8.37% of protein as determined by the Kjeldahl method (9).

General Method.—The general method of extraction of the plant material by a series of organic solvents (10) was applied to 50 Gm. The petroleum ether extract contained a plant wax and a small quantity of material tentatively identified as a fixed oil or an aromatic ester. The ether extract contained a resin. The chloroform extract yielded additional resin. The alcohol extract contained tannins, carbohydrates and reducing substances. The ethyl acetate extract yielded a very small quantity of phlobaphenes.

* The original thesis contains 35 photomicrographs of the plant sections.

Petroleum Ether Extract.—About 75 Gm. of the powdered plant material were extracted with petroleum ether in a Soxhlet extractor. The plant material (*A*) was reserved for use in the lead method. The petroleum ether extract contained a wax and a fixed oil or an aromatic ester which upon treatment with alkali and acid yielded an organic acid with a melting point of 38.0° C.

Lead Method.—The lead method (11) for the separation and identification of the hot-water soluble constituents was applied to *A*. The precipitate obtained with lead acetate contained tannins and mucilages or pectins; the precipitate from basic lead acetate contained tannins and either mucilage or pectin or both and gave indications of the presence of glycosides; the filtrate from the lead precipitates gave tests for reducing substances and carbohydrates but no tests for glycosides or alkaloids.

Pectin and Mucilage.—A decoction of the powdered plant material was diluted with alcohol until the concentration of the alcohol in the mixture was 70%. The stringy, mucilaginous, brown precipitate which separated was filtered out and purified. The presence of pectin in this purified material was indicated by jelly formation with sucrose (12) and precipitation from aqueous solution with calcium chloride T.S. (13). The presence of either pectin or mucilage or both was indicated by the presence of methyl alcohol after hydrolysis (13), the presence of calcium and magnesium in the ash (14), precipitates formed in aqueous solution with certain reagents (15) and the production of mucic acid upon oxidation (16).

The powdered plant material contained 0.626% of pectin weighed as calcium pectate (17). The approximate factor 2.041 was developed experimentally to convert calcium pectate to equivalent pectin. When multiplied by 0.626%, it gave 1.28% of pectin in the plant material.

The 70% alcohol filtrate from the precipitation of pectin and mucilage was detannated with magnesium oxide. The filtrate contained reducing substances and carbohydrates which yielded an osazone with a melting point of 195.5° C., a value conforming to that for galactosazone. The filtrate gave no test for pentose sugars (18).

Alcoholic Extract.—About 3 Kg. of the powdered plant material were extracted with alcohol in a Barnstead extractor, the liquid extract was evaporated to dryness and the residue was designated as "Barnstead extract."

A portion of this "Barnstead extract" was extracted with boiling alcohol, followed by boiling water. The alcohol extract contained a wax with a melting point of 69.8° C. and a resin, and gave tests for carbohydrates and reducing substances. The brown aqueous extract gave tests for tannins, carbohydrates and reducing substances. Hydrolysis with hydrochloric acid of aqueous solutions of the residues from the evaporation of both of the liquid extracts produced no change in optical rotation or no increase in copper reducing action when determined by the method of Shaffer-Somogyi (19). This indicated the absence of a glycoside. The portion of the "Barnstead extract" which was insoluble in alcohol and water consisted largely of phlobaphenes, and an ash of a portion gave tests for phosphates, magnesium, calcium, sodium and potassium.

General Method: A portion of the semi-solid "Barnstead extract" was mixed with an equal weight of clean, dry sand and this mixture was subjected to extraction (10) by a series of organic solvents. The petroleum ether extract yielded a plant wax and a semi-solid fatty material which upon saponification and acidification of the alkaline mixture gave an organic acid with a melting point of 41.5° C. The ether extract contained a small quantity of a brittle, brown solid which appeared to be a resin of indefinite melting point. The chloroform extract yielded a small quantity of a solid which appeared to be the same as that obtained from the ether extract. The alcohol extract contained tannins, phlobaphenes and carbohydrates and the ethyl acetate extract left a residue of tannins and phlobaphenes. An aqueous extract yielded a small quantity of tannins and carbohydrates.

Aromatic Ester, Sterol and Resin: The remainder of the "Barnstead extract" was extracted with boiling alcohol and the liquid extract was concentrated and refrigerated to remove wax. This was filtered out, the filtrate was absorbed upon purified oak sawdust and the mixture was oven dried. The dry product was extracted in a Soxhlet with petroleum ether followed by ethyl alcohol.

The petroleum ether extract was evaporated to dryness. The semi-solid, green-black residue (*O*) had an acid value of 50.86, a saponification value of 151.26 and an ester value (calculated) of 100.40. An ether solution of a portion of the residue was extracted with 1% ammonium carbonate (*B*), 1% sodium carbonate (*C*), 0.1% sodium hydroxide (*D*) and 1% sodium hydroxide (*E*) solutions (20). The ether solution which remained was labeled *M*. Acidification of the alkaline extracts yielded an organic acid in each case. The acids from *B*, *D* and *E* were too small in quantity to permit of a melting point determination. The acid from *C* had a melting point of 51.0° C.

Ether solution *M* was evaporated to dryness, the dark green residue was refluxed with potassium hydroxide solution and the alkaline solution was extracted with ether. The residue from this ether extract gave no tests for sterols or for alcohols although an alpha-naphthyl urethane derivative (m. p. 296.8° C.) was obtained. The alkaline solution was next acidified and extracted with ether. This ether extract contained an organic acid with a melting point of 42.4° C.

Another portion of the petroleum ether residue (*O*) was distilled in a Hickmann vacuum still. The distillate contained a wax, a sterol and a brownish black ester which yielded an organic acid. The oily, pale yellow distillate had an acid value of

49.47, an ester value of 113.1 and a saponification value (calculated) of 162.6. The ether extract of a saponified mixture yielded a sterol and a yellow, semi-solid alcohol which had an odor resembling nutmeg. An ether extract of this saponified mixture after it had been acidified, contained an organic acid with a neutralization equivalent of 297.6. The acid formed a *p*-bromophenacyl bromide ester with a melting point of 69.0° C. The aqueous-acid solution after extraction with ether did not contain glycerin, indicating that the distillate did not contain a glyceryl ester (21).

The dark green alcohol extract from the sawdust mixture was concentrated in volume and diluted with 5 volumes of ether to precipitate tannins, sugars, etc. The precipitate was filtered out and the filtrate, which contained resins, was evaporated to dryness. The residue, considered to be resin, had an acid value (quinhydrone electrode) of 2.17, a saponification value (glass electrode) of 235.8 and an ester value (calculated) of 233.6.

An ether solution of this residue was extracted with 1% ammonium hydroxide (*F*), 1% sodium carbonate (*G*), 0.1% sodium hydroxide (*H*) and 1% sodium hydroxide (*I*) solutions. The ether solution which remained was labeled *N*. Acidification of the alkaline extracts yielded organic acids, the quantity from *I* being too small for tests. The acid from *E* had a melting point of 137–144° C., that from *G* a melting point of 186.6° C. and formed an ethyl ester with a melting point of 152.4° C. and that from *H* a melting point of 196–201° C. and formed a *p*-bromophenacyl bromide ester with a melting point of 151–171° C. Ether solution *N* was evaporated and the residue was saponified. Ether removed from this saponified mixture a small quantity of a substance which would not give a test for the hydroxyl group or would not form a derivative. Extraction with ether of this saponified mixture after it had been acidified, yielded an organic acid (m. p. 54.7° C.) which would not form the usual derivatives.

PHARMACOLOGICAL*

A fluidextract prepared by Type Process A of the U. S. P. produced a lowering in the blood pressure of a rabbit. When administered orally with the ration, it had no beneficial effect upon Pneumococcus Type II infections in mice. It had a bacteriostatic effect upon 4 of a series of 12 microörganisms *in vitro*. That this action resided in the tannin was indicated by the fact that a detannated fluidextract had no bacteriostatic effect upon any of the 12 microörganisms.

*The pharmacological and bacteriological tests were conducted by Dr. Raymond Bieter, Dr. Winfred P. Larson, Milton Levine, Elizabeth M. Cranston and Charles Drake of the School of Medicine, University of Minnesota.

SUMMARY

The histological investigation showed a structural arrangement typical of the dicotyledons with secondary growth. The abaxial surfaces of the leaves had two types of uni-cellular, non-glandular hairs.

The phytochemical investigation showed the presence of a catechol or catechol-phloroglucin tannin, a bitter substance, starch, pectin, mucilage, sterols, an aromatic ester, a wax, an ester-resin, small quantities of resin-acids and galactose. No alkaloids or glycosides were found. The total and acid-insoluble ash, moisture and some of the constants of the ester and resin were determined as well as the quantity of protein and tannin and an approximation of the quantity of pectin and mucilage.

CONCLUSION

Purported curative action obtained by the Indians from infusions, decoctions and poultices of this plant was probably due to the bacteriostatic action of the tannins and the demulcent effect of the mucilage and pectin.

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A Comparative Study of the Total Volatile Acids of Viburnum Stem and Root Barks*

By Irvine W. Grote and Charles Colburn†

Two species of Viburnum are official in N. F. VI, namely, *V. prunifolium* and *V. opulus*. The latter is a small shrub and since its introduction into U. S. P. VII the "dried bark" has been official. *V. prunifolium* was official in U. S. P. VI and VII as the "dried bark." In U. S. P. VIII, the root bark was specified, but this was changed back to the "dried bark" in U. S. P. IX and again changed to "root bark" in N. F. V and VI. In U. S. P. VIII and IX, *V. lentago* could be used as *V. prunifolium*. Examination of the literature does not reveal any scientific reason for this alternate change from *V. prunifolium* whole bark to root bark. Continued collection of the root bark of *V. prunifolium* will lead to a growing scarcity, while, if merely the stem bark is collected, the tree is quickly replaced by shoots from the original root system. This paper represents the first in a series of comparisons by chemical means of the stem and root barks of *V. prunifolium*.

Both Valerian and Viburnum are peculiar in that they are reported to contain large amounts of valeric acid compounds as well as unusually large amounts of other volatile aliphatic acids released upon hydrolysis. We have found in our own laboratory that the bark of the various Viburnums release several times as much volatile acid following hydrolysis as do such barks as *Cascara Sagrada*, etc. Many workers have assumed that the valeric acid compounds are responsible for the uterine sedative action of the two drugs. Heyl and Barkenbus (1) made perhaps the most careful study of *V. prunifolium* root bark and report after hydrolysis the presence of the volatile acids formic, acetic, valeric and traces of butyric.

No simple direct methods for accurate quantitative determinations of the separate aliphatic acids in presence of several others of the same series could be found in the literature. The steam distillation method of D. C. Dyer (2), however, seems to afford a useful and rapid method of comparison of the amount of one mixture of volatile acids with another and to determine any marked variation in composition of the acids in the mixture. The method consists of steam distillation of the acids from a constant volume of solution and titration of successive 100-cc. fractions of the distillate with alkali. Each of the lower aliphatic acids steam distils at a constant rate proportional to the amount of volatile acid still present in the constant volume. The rate for the acids, through caproic at least, is characteristic for the individual acid and is the reverse of what would be expected, caproic acid distilling at the most rapid rate and formic acid at the slowest rate. Mixtures of the acids distil at rates proportional to the amount of each acid present in the mixture.

In view of the apparent importance of the volatile acids in Viburnum bark, and their possible relation to physiological action, it seemed worth while to apply Dyer's method in a comparison of stem and root barks of *Viburnum prunifolium*. The method was modified in that no attempt was made to keep the volume of liquid constant but rather to run the distillations under identical conditions. In addition, Dyer has shown that over twenty successive 100-cc. fractions were required to remove substantially all the formic acid. This would make the method unnecessarily long for comparative purposes. By the fifth successive 100-cc.

* Presented before the Scientific Section, A. PH. A., Richmond meeting, 1940.
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